bio-protocol Preprint

Not Peer Reviewed
Ask a question online

ssDRIP-seq and DRIP-qPCR

Yaoyi Li Wei Xu Yawei Song Qin Li Qianwen Sun Hongjie Yao

Updated date: Nov 2, 2020

An abbreviated version of this protocol was published in Science Advances in Jun 2020

R-loops coordinate with SOX2 in regulating reprogramming to pluripotency

DOI: 10.1126/sciadv.aba0777

Detailed protocol

gDNA extraction

Resuspend the pellet in TE buffer (1.6~6.4 ml, depending on the quantity of pellet) and add SDS (final concentration: 0.5%) and Proteinase K (final concentration: 0.1 mg/ml). Then the mixture was incubated at 37°C in a shaker with 180~240 rpm for 4~12 hours.

Add 1/4 volume 5 M KAc, mix and place the tube on ice, 10~20 min.

Add 1 volume phenol:chloroform:isoamyl alcohol (25:24:1), mix well by Vortex, and centrifuge at 12000 g for 10 min at 4°C.

Transfer the supernatant to a new tube, add 1 volume chloroform:isoamyl alcohol (24:1), and mix well by vortex. Centrifuge at 12000 g for 10 min at 4°C.

Transfer the supernatant to a new tube, and centrifuge again at 12000 g for 5 min at 4°C. Transfer only 90% of the supernatant.
Precipitate the supernatant by adding 0.7 volume isopropanol, and incubate for 30 min at room temperature. Centrifuge at 12000 g for 10 min at 4°C and wash the pellet with 1 ml 70% ethanol. Air dry the pellet and resuspend with 200~500 µl ddH2O.

Cocktail of restrict enzymes digestion

Digest 2~10 µg gDNA in 200 µl final volume, 8~16 hours, by RE combinations. MseI, DdeI, AluI, and MboI (final concentration: 100 U/ml for each enzyme). Incubate at 37°C overnight.

Add 1 volume phenol:chloroform:isoamyl alcohol (25:24:1), mix well by Vortex, and centrifuge at 12000 g for 10 min at 4°C.

Transfer the supernatant to a new tube, add 1 volume chloroform:isoamyl alcohol (24:1), and mix well by vortex. Centrifuge at 12000 g for 10 min at 4°C.

Transfer the supernatant to a new tube, and centrifuge again at 12000 g for 5 min at 4°C. Transfer only 90% of the supernatant.
Precipitate the supernatant by adding 0.7 volume isopropanol, and incubate for 30 min at room temperature. Centrifuge at 12000 g for 10 min at 4°C and wash the pellet with 1 ml 70% ethanol. Air dry the pellet and resuspend with 200~500 µl ddH2O.
Fragmented gDNA was quantified by Qubit.

DRIP

Take 10 µg fragmented gDNA to a 1.5 ml Safe-lock EP tube and add 50 µl 10X DRIP binding buffer (100mM NaPO4 pH7.0, 1.4M NaCl, 0.5% Triton X-100), and 10 µg S9.6 antibodies. (store the rest gDNA as input DNA for input-library or input of qPCR).
Incubate for 8~16 hours at 4°C while gently inverting on a rotisserie shaker.

50 µl Dynabeads (Protein A or G) are used for each tube. Beads are pre-washed with 1X DRIP binding buffer (10x diluted in TE) for 3 time, and each wash take 5~10 min with gentle shaking at 4°C.

Add DNA/S9.6 complexes to pre-washed beads, and incubate with gentle shaking at 4°C for 3~4 hours.

Wash 4 times beads/antibody complexes (remove background) with 1X DRIP binding buffer at room temperature, and each wash takes 5~10 min.

Add 250 µl elution buffer (50mM Tris pH8.0, 10mM EDTA) and 200 µg Proteinase K (10 µl) to beads/antibody complexes, incubate for 60 min in an Eppendorf ThermoMixer at 55°C, 1000 RPM.

Cleanup elution with 250 µl phenol/chloroform extraction, and move supernatant to a new tube. Then add 1/10 volume 3 M NaAc, 1 µl glycogen and 2.5 volume ethanol. Precipitate the DNA at -20°C for 2 hours.

ssDRIP-seq library was constructed by using the Accel-NGS 1S Plus DNA Library Kit (Swift Biosciences).

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Li, Y. , Xu, W. , Song, Y. , Li, Q. , Sun, Q. and Yao, H. (2020). ssDRIP-seq and DRIP-qPCR. Bio-protocol Preprint. bio-protocol.org/prep597.
2. Li, Y., Song, Y., Xu, W., Li, Q., Wang, X., Li, K., Wang, J., Liu, Z., Velychko, S., Ye, R., Xia, Q., Wang, L., Guo, R., Dong, X., Zheng, Z., Dai, Y., Li, H., Yao, M., Xue, Y., Schöler, H. R., Sun, Q. and Yao, H.(2020). R-loops coordinate with SOX2 in regulating reprogramming to pluripotency . Science Advances 6(24). DOI: 10.1126/sciadv.aba0777

Copyright: Content may be subjected to copyright.